The Efficacy of Recombinant TPO in Murine and Nonhuman Primate Models for Myelosuppression and Stem Cell Transplantation

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ABSTRACT

Radiation-induced pancytopenia proved to be a suitable model system in mice and rhesus monkeys to study thrombopoietin (TPO) target cell range and efficacy. TPO was highly effective in rhesus monkeys exposed to the midlethal dose of 5-Gy (300 kV x-rays) TBI, a model in which it alleviated thrombocytopenia, promoted red cell reconstitution, accelerated reconstitution of immature CD34$^-$ bone marrow (BM) cells and potentiated the response to growth factors such as GM-CSF and G-CSF. The accelerated reconstitution of BM CD34$^+$ cells appeared to be reflected by a similar rise in peripheral blood CD34$^+$ cells, both being augmented by concomitant GM-CSF. However, TPO was ineffective following transplantation of limited numbers of autologous BM or highly purified stem cells in monkeys conditioned with 8-Gy TBI. In the 5-Gy model, a single dose of TPO 24 h after TBI was effective in preventing thrombocytopenia and was augmented by GM-CSF. The strong erythropoietic stimulation may result in iron depletion and TPO treatment should be accompanied by monitoring of iron status. In mice, similar observations were made and the importance of dose and dose schedule for stimulation of multilineage repopulating cells versus the lineage-dominant thrombopoietic response studied in detail. *Stem Cells 1998;16(suppl 2):127-141*

INTRODUCTION

The identification of thrombopoietin (TPO) [1-4] as the major regulator of thrombocyte production [5, 6] has resulted in novel insights in the regulation of immature hemopoietic cell differentiation [7-9] and potentially provided a therapeutic approach to counteract thrombocytopenic states, in particular those associated with intensive cytoreductive treatment of malignancies. Its pharmaceutical development for the latter application requires demonstration of efficacy in experimental animal models, alone and in conjunction with other cytokines. In view of the generally complex receptor distribution patterns of

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EXHIBIT 6

growth factors [10-12], interactions resulting from concurrent administration of the growth factors are difficult to predict by any other approach than detailed experimental animal in vivo studies.

Myelosuppression is a serious complication of current chemotherapy regimens resulting in life-threatening neutropenia and thrombocytopenia and hampering full deployment of anticancer therapy. Both G-CSF and GM-CSF treatment have become established therapeutics [13-17] to alleviate the cytopenia, in particular the neutropenia resulting from intensive cytoreductive treatment [18]. Although G-CSF and GM-CSF are grossly similar in pharmaceutical profile [18, 19], GM-CSF has advantages in that it also stimulates megakaryocytopoiesis and monocyte differentiation [20, 21]. The beneficial effects of both GM-CSF and G-CSF on neutropenia following cytoreductive treatment are in most studies restricted to an approximate five days earlier recovery or less in dose-intensified chemotherapy [15, 17, 22] on a total median neutropenia of about 20-25 days. It is, therefore, of considerable importance to select combinations of growth factors that provide optimal costimulatory efficacy.

Radiation is an effective and controllable single agent to induce myelosuppression, and is the most effective single agent for high-dose eradicative treatment and immunosuppression. For the preclinical evaluation of TPO, we have made use of rhesus monkeys exposed to the midlethal dose of 5-Gy TBI (300 kV x-rays), as well as the lethal dose of 8-Gy TBI followed by an autologous transplant of highly purified stem cells. The 5-Gy TBI dose results in a profound pancytopenia for three weeks, whereas 8-Gy irradiated monkeys need a transplant to prevent protracted pancytopenia and mortality due to transfusion refractoriness. Our study parameters included, apart from blood cell counts, assessment of immature bone marrow (BM) cells as well as monitoring of adverse effects. To assess combination treatment, priority was given to those growth factors which are likely to be used clinically in conjunction with TPO, i.e., GM-CSF and G-CSF. In this summary paper, we focus on the myelosuppression data and mechanisms of action promoting efficacy, and briefly discuss the transplantation experiments. The findings in rhesus monkeys prompted us to return to more basic studies in mice to elucidate some of the relevant mechanisms. In mice, we calibrated the 5-Gy x-rays of the monkeys to 6-Gy γ-rays to accomplish a similar level of myelosuppression in keeping with the relative biological effectiveness of γ-rays and a similar duration of pancytopenia.

Thrombopoietin as a Single Agent in Myelosuppressed Rhesus Monkeys: Prominent Thrombopoietic and Erythropoietic Stimulation with Iron Depletion, Lack of a Neutrophil Response, and Accelerated BM CD34$^+$ Cell Recovery

The effectiveness of TPO in alleviating thrombocytopenia was initially evaluated in a placebo-controlled study involving rhesus monkeys exposed to 5-Gy TBI using supraoptimal treatment with human recombinant TPO (10 μg/kg/day s.c., day 1-21 after TBI) [23, 24]. The TPO treatment appeared to be highly effective in preventing thrombocytopenia, with nadirs for thrombocytes on average far above 100×10^9/l, a greatly accelerated recovery to normal values and no need for thrombocyte transfusions, whereas placebo controls needed two to six platelet transfusions. TPO appeared to act selectively in that neutrophil regeneration was not influenced, but the red cell lineage recovery was prominently stimulated, exponential reticulocyte regeneration being initiated 10 days earlier than in placebo-treated animals. The reticulocytosis was followed by a normoblastosis which occurred earlier, was more pronounced than in placebo-treated monkeys and was accompanied by elevated lactic dehydrogenase serum levels, attributed to a rapid and partly inefficient erythropoiesis. The effect of TPO on the red cell lineage was also reflected in a less profound nadir for hemoglobin and hematocrit values than in placebo controls. Simultaneous TPO and G-CSF were as effective as TPO in preventing thrombocytopenia, although platelet levels did not rise to the supranormal levels seen with TPO alone. The neutrophil recovery was greatly augmented compared to G-CSF treatment alone, resulting in a less profound nadir and a recovery that started much earlier, as was similarly observed for monocyte, CD11b$^+$, CD16$^+$ and CD56$^+$ cell reconstitution. In addition, TPO strongly promoted the recovery of BM cellularity and

granulocyte/macrophage and erythroid progenitor cells, as was also reflected by a two orders of magnitude difference with controls in BM $CD34^+$ cells in the second week of treatment, whereas G-CSF had no influence. There was no effect of TPO on hemostasis parameters.

The study thus showed that TPO is highly effective in preventing thrombocytopenia following radiation-induced myelosuppression, an effect characterized by alleviation of platelet nadirs, accelerated recovery, and a substantial reduction in the time needed to reach normal platelet levels. TPO also stimulated red cell reconstitution and was selective in that neutrophilic granulocytes, monocytes and lymphocytes were not influenced. In addition, TPO markedly promoted immature $CD34^+$ BM cell reconstitution as was also reflected in increased numbers of immature progenitor cells along the granulocyte/macrophage and erythroid lineages. Simultaneous administration of G-CSF did not influence TPO-stimulated recovery of platelets, although platelet levels in the third week of treatment tended to be lower than in the monkeys treated with only TPO. A similar dampening effect of G-CSF on the TPO response has been described in mice [25] and might perhaps be related to the protracted thrombocytopenia observed in stem cell-transplanted monkeys [26] which were treated with G-CSF. Remarkably, TPO treatment considerably augmented G-CSF-stimulated neutrophil and monocyte reconstitution. Adverse systemic effects were not observed for either growth factor.

The thrombopoiesis-stimulating effect of TPO was already sufficiently effective in the first week of treatment, as was clear from the increasing platelet numbers as early as day 8, as opposed to placebo controls which did not reach similar levels before the fourth week after TBI. Also, the effect on BM cellularity and immature BM cells is already clear at the end of the second week of treatment. Therefore, the dose schedule of 21 consecutive days of treatment was supraoptimal, as was also clear from the supranormal platelet numbers reached in the second and third week after TBI. It was previously shown in a mouse model of thrombocytopenia that a single dose of TPO given 24 h after the cytoreductive therapy is as effective as daily dosing for eight consecutive days [27]. The effect of single injections in this model was also highly dose-dependent. TPO was clearly more effective in stimulating platelet recovery than other growth factors known to stimulate platelet production such as interleukin 6 (IL-6) [28-31], IL-11 [32, 33], IL-3 [30, 31], and IL-1 [34]. All of these cytokines stimulate platelet production, but are not sufficiently effective in preventing thrombocytopenia at tolerable doses in view of the adverse effects observed.

A central issue of TPO treatment is prevention of bleeding as a consequence of myelosuppression. Our policy to transfuse donor thrombocytes at the level of 40×10^9/l coincides with the first appearance of petechiae and other bleeding and was chosen to prevent undue deaths due to hemorrhages. For obvious reasons, this level is higher than used for human patients, where instructions can be given and sensitization to alloantigens should be avoided. However, as can be extrapolated from the postirradiation drop in thrombocyte counts after the first week and the first ascending counts in the third week after TBI in placebo-treated monkeys and has also been apparent in historical controls [35-37], without transfusions the thrombocytes would have dropped to levels lower than 10×10^9/l within two days after the first transfusion. Thus, it can be concluded that the TPO treatment, which prevented the decline of thrombocyte counts to levels lower than 100×10^9/l early in the second week after irradiation, also effectively prevented the propensity to bleeding.

The TPO effect on early hematopoietic progenitor cells of different lineages was unexpected, but is in line with other observations [38, 39] and consistent with the observation that its receptor is present on immature progenitor cells [7]. As megakaryocytes are capable of releasing various growth factors [40, 41], the effect observed may be an indirect consequence of stimulation of the megakaryocyte lineage. Alternatively, TPO may synergize directly with growth factors such as kit ligand or flt-3 ligand to stimulate immature cells. Evidence for such a synergy has been provided by in vitro experiments [42-44]. The accelerated recovery of progenitor cells is perhaps best described by a two-log increased reconstitution of immature $CD34^+$ cells in the BM aspirates of TPO-treated monkeys at the end of the second week of treatment. In magnitude, this effect surpassed that of any other growth factor tested in the same animal model,